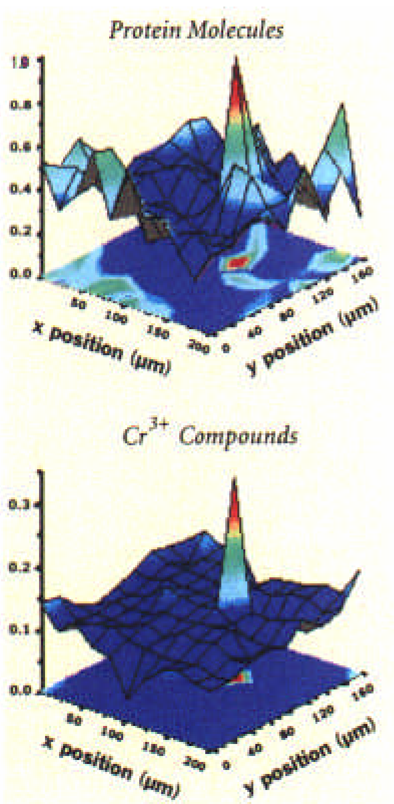
Infrared Beamline Reveals Toxin-Reducing Microbes

Lawrence Berkeley Lab scientists have discovered a bacteria that thrives on toxic waste. A Fourier-transform infrared (FTIR) spectromicroscopy beamline (Beamline 1.4.3) was implemented to identify the toxin-devouring microbes. The bacteria detected converts hexavalent chromium (Cr^{6+}) a widespread industrial contaminant, into a safer, stable compound (Cr^{3+}). “Basically, the bugs eat the waste,” said Paul Preuss, a spokesperson for Lawrence Berkeley Lab.

Hoi-Ying Holman, along with colleagues Dale Perry, Michael Martin, Wayne McKinney, and Jennie Hunter-Cevera, made the discovery by examining core samples from 75 meters beneath a toxic waste containment site in Idaho. By using infrared spectromicroscopy, they were able to follow the reduction of toxic material among populations of living organisms on minerals: Lawrence Berkeley reports this is die first time that biogeochemical transformation of Cr^{6+} on a mineral surface has been nondestructively monitored and studied.



Spatially resolved infrared spectromicroscopy of a basalt surface shows a strong correlation between concentrations of biological molecules characteristic of microbes (*top*) and peak concentrations of reduced chromium (*below*).

Since infrared light does not kill bacteria, the transformation of Cr^{6+} could be monitored as it occurred. Distinct infrared absorption bands served dual functions--as chemical markers to detect different chromium species, and as biological markers to detect the presence and activity of microorganisms on specimen surfaces. The brightness of the infrared radiation from the beamline also made spatially resolved spectromicroscopy possible.

Previously, two mechanisms for reducing Cr^{6+} compounds were postulated. The biological mechanism requires the presence of microorganisms to aerobically reduce the Cr^{6+}. The chemical mechanism relies on metal oxides, such as Fe(II) compounds, to catalyze the Cr^{6+} reduction reaction. Working with an infrared beamline over a five-day period, the researchers applied Fourier transform spectromicroscopy to observe the steps in the reduction process and the precise location of the reduced chromium. In addition, the effects on the biotic reduction process of common organic co-contaminants, such as toluene vapor, were evaluated.

“The infrared is the end of the spectrum not usually associated with synchrotrons,” said Holman, “but for us it's perfect--and not only because it's non-destructive of organisms. You have an extremely complicated spectrum in the 10 µm region [the dimension of the beam]. We identified markers in this spectral region that tracked the key compounds that undergo changes. We could resolve the spectrum in time, to follow the different steps of the reduction, and also in space, to see exactly where the reactions were happening.”

On the magnetite samples with no living bacteria, no significant changes were evident. On samples with living microorganisms *(Arthrobacter oxydans)* in the absence of toluene, a weak chromium reduction was detected. However, when the *Arthrobacter oxydans* had been exposed to toluene, FTIR spectromicroscopy revealed that hexavalent chromium and toluene had been replaced by pentavalent chromium and hydrocarbon degradation in association with biomolecules. This reduction of Cr^{6+} occurred directly at the sites of bacterial concentration. Imaging the surface at characteristic absorption bands demonstrated a strong correlation between peak depletion of Cr^{6+} and toluene, and peak concentration of biological molecules.

To determine if this microbial reduction process could occur in real geologic samples, Holman exposed composite mineral surfaces of basalt rock chips containing resident communities of microbes to solutions of Cr^{6+} and toluene vapor. After four months, FTIR spectromicroscopy showed that chromium-tolerant and chromium-reducing natural microorganisms were thriving in association with Cr^{3+} (see figure).

“As far as we know,” says Holman, “this is the first time that infrared synchrotron studies have been used to follow the steps in the transformation of toxic chromium on mineral surfaces.”

Although there are other microbes known to transform toxic heavy metals, *Arthrobacter oxydans* is the first known type of bacteria able to live deep underground. Holman's research has uncovered a way to monitor toxic waste disposal which could represent an advance over current methods. The FTIR method can now be expanded to examine other infrared-amenable microbial/chemical contaminant systems. Says Holman, “This should help in the design and implementation of new, environmentally benign remediation techniques for cleaning up mixed-waste sites.”